REMARKS

Status of the claims

Claims 1-19 are presently pending in the application. Claims 1-4 and 7-19 have been withdrawn from consideration and examined claims 5 and 6 stand rejected under 35 U.S.C. §§ 112 and 102. Claims 5 and 6 have been amended herein in a sincere effort to improve clarity. New claim 20 has been added. In addition, the specification has been amended to correct typographical errors. Entry of the foregoing amendments and consideration of the following remarks is respectfully requested.

Drawings

The drawings were objected to because reference characters in Fig. 1, Fig. 2 and Figs. 3A-C allegedly did not correspond to additional references at pages 29, 31 and 32, respectively. By amendment herein, Applicants have removed the references to these Figures at pages 29, 31 and 32, thereby obviating this objection.

Applicants further note that the originally submitted specification contained nine Figures as set forth in the substitute specification. However, because the originally submitted specification referred to more than one Figure by the same reference number (*e.g.*, there were multiple Figure 1s), the Figures were renumbered for clarity in the substitute specification.

Information Disclosure Statement

Applicants acknowledge with appreciation that the IDS submitted on July 9, 2002 is under consideration by the Examiner.

Specification

A) Sequence Listing: A corrected Sequence listing is submitted herewith.

B) Abstract/Background: The Examiner indicates confusion in regards to two abstracts and/or two background sections. Applicants note that the original application included manuscripts with their own "Abstract" and "Background" sections. To clarify this confusion, Applicants submitted the current substitute specification, which contains only one abstract and one background.

C) Embedded Hyperlinks: By amendment herein, the embedded hyperlinks in the specification have been removed.

D) Status of Related Applications: As requested by the Examiner, the paragraph regarding the status of related applications has been corrected by amendment herein.

E) Typographical errors: Applicants acknowledge with appreciation the typographical errors cited by the Examiner and have corrected those errors by amendment herein. In addition to the typos noted by the Examiner, Applicants have corrected a typographical error on page 11, line 9 by amendment herein.

F) Reference Numbers: Applicants wish to clarify that all the numbers in parentheses in Example 1, pages 28-29, are citations to the "references cited in Example 1," as found on page 39-40.

35 U.S.C. § 112, second paragraph

Claims 5 and 6 are rejected as allegedly indefinite. (Office Action, page 8). In particular, the terms "first and second" are alleged to be unclear. Further, the term "complex" in claim 5 is alleged to be unclear in that it is allegedly used as both a noun and a verb. In addition, the metes and bounds of the term "nonnaturally occurring peptides" are alleged to be not clearly set forth. (Office Action, page 9). Finally, the recitation "wherein the first and second peptide linkers are first and second copies of the same linker" is alleged to be confusing and lack antecedent basis. (Office Action, page 9).

Although Applicants submit that the claims were sufficiently clear and definite as filed, claims 5 and 6 have been amended herein to remove (1) the terms "first and second" and (2) the term "complex" where it was used as a verb.

With regard to the term "non-naturally occurring," Applicants direct the Examiner's attention to the paragraph beginning on line 3 of page 8, where the term is clearly defined as sequences not found in nature. In other words, natural linker sequences are plainly excluded from the scope of the pending claims.

In view of the foregoing amendments and remarks, Applicants request withdrawal of the rejections based on 35 U.S.C. 112, second paragraph.

35 U.S.C. 102

Claims 5 and 6 stand rejected as allegedly anticipated by various cited references. Applicants address each reference in turn.

Eisenberg

Claim 5 stands rejected as allegedly anticipated under 35 U.S.C. 102(e) by U.S. Patent No. 6,453,242 (hereinafter "Eisenberg"). (Office Action, page 10).

Eisenberg is not directed to the claimed compositions in which the at least two fusion proteins are joined via specific binding between peptide linkers, where each fusion protein comprises a peptide linker. Rather, Eisenberg is directed entirely to the use of a single (usually naturally occurring) peptide linker that mediates linkage of the zinc finger proteins. (See, column 10, lines 20-59 of Eisenberg). There is no disclosure in Eisenberg regarding the use of multiple peptide linkers that interact with each other to mediate binding of two molecules. Indeed, no such suggestion can exist in Eisenberg (or any of the references) because, prior to the present disclosure, it was thought that one peptide linker would suffice. Applicants were the first to attempt and the first to successfully demonstrate that dimerizing peptides could be linked to each other as claimed. Accordingly, Eisenberg fails to disclose or suggest elements of the pending claims and withdrawal of this rejection is requested.

Choo and Liu

Claim 5 stands rejected as allegedly anticipated under 35 U.S.C. 102(a) WO 98/53058 (hereinafter "Choo"). (Office Action page 11). In addition, claims 5 and 6 stand rejected as allegedly anticipated under 35 U.S.C. 102(b) by Liu et al. (1997) *Proc. Natl. Acad. Sci USA* 94:5525-5530. Liu and Choo are cited for disclosing the naturally occurring amino acid linkers, TGE(Q)KP, to link zinc finger proteins to each other.

As noted above, the pending claims are directed to fusion molecules that include at least two, nonnaturally occurring peptide linkers. In contrast, Liu and Choo parrot the conventional wisdom at the time the application was filed, namely that a single 5 amino acid linker should be used to link ZFPs. (see, *e.g.*, page 5528 of Liu noting that "[c]omparative studies of the constructed TGEKP linker with the natural linkers observed in the Zif268 structure indicated that this linker as optimal a linker peptide as any novel linker sequence that could be designed."). Therefore, Liu and Choo actually teaches away from Applicants' complexes and, accordingly, this reference does not anticipate the pending claims.

Kim

Claim 5 stands rejected as allegedly anticipated under 35 U.S.C. 102(b) by Kim et al. (PNAS, 1998) (hereinafter "Kim"). (Office Action page 12).

Because Kim fails to disclose or suggest key elements of the claims as pending, Applicants traverse this rejection.

Pending claim 5 is drawn to complex comprising two or more fusion proteins, each fusion protein comprising a ZFP and a peptide linker. In other words, at least 2 ZFPs are complexed together and at least two peptide linkers are present.

Like the other references, Kim contains absolutely no disclosure regarding linking two fusion proteins together where each protein contains its own peptide linker. Accordingly, there is no disclosure or even suggestion in this reference to arrive at the claimed complexes. Since Kim fails to describe, demonstrate or suggest the use of the claimed linkers, Applicants submit that withdrawal of this rejection is in order.

In sum, none of the cited references describe, teach or suggest compositions as claimed by Applicants.

CONCLUSION

Applicants believe that the claimed subject matter is now in condition for allowance and early notification to that effect is respectfully requested. If any issues remain to be addressed, the Examiner is encouraged to telephone the undersigned.

Please address all correspondence to the undersigned.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Please amend the paragraph beginning on line 23 of page 1 as follows:

--The x-ray crystal structure of Zif268, a three-finger domain from a murine transcription factor, has been solved in complex with a cognate DNA-sequence and shows that each finger can be superimposed on the next by a periodic rotation. The structure suggests that each finger interacts independently with DNA over 3 base-pair intervals, with side-chains at positions -1, 2 , 3 and 6 on each recognition helix making contacts with their respective DNA triplet subsites. The amino terminus of Zif268 is situated at the 3' end of the DNA strand with which it makes most contacts. ~~DNA recognition subsite.~~ Recent results have indicated that some zinc fingers can bind to a fourth base in a target segment (Isalan et al., *PNAS* 94, 5617-5621 (1997)). If the strand with which a zinc finger protein makes most contacts is designated the target strand, some zinc finger proteins bind to a three base triplet in the target strand and a fourth base on the nontarget strand. The fourth base is complementary to the base immediately 3' of the three base subsite.--

Please amend the paragraph beginning on line 19 of page 2 as follows:

--A number of papers have reported attempts to produce ZFPs to modulate particular target sites. For example, Choo et al., *Nature* 372, 645 (1994), report an attempt to design a ZFP that would repress expression of a brc-abl oncogene. The target segment to which the ZFPs would bind was a nine base sequence ~~5'GCA GAA3' GCC~~ 5' GCA GAA GCC3' chosen to overlap the junction created by a specific oncogenic translocation fusing the genes encoding brc and abl. The intention was that a ZFP specific to this target site would bind to the oncogene without binding to abl or brc component genes. The authors used phage display to screen a mini-library of variant ZFPs for binding to this target segment. A variant ZFP thus isolated was then reported to repress expression of a stably transfected brc-able construct in a cell line.

Please amend the paragraph beginning on line 17 of page 3 as follows:

--The present application is related to copending applications 09/229,007 filed January 12, 1999 (WO 00/42219), now U.S. Patent No. 6,453,242 and 09/229,037 filed January 12, 1999 (WO 00/41566), and both incorporated by reference in their entirety for all purposes.--

Please amend the paragraph beginning on line 33 of page 3 as follows:

--The invention further provides methods of selecting a dimerizing peptide. Such methods entail providing a phage display library in which a member displays a zinc finger protein fused to a peptide from its outersurface, the zinc finger protein being the same in different members, and the peptide varying between different members. The library is then contacted with a nucleic acid substrate comprising first and second binding sites for the zinc finger protein. Phage displaying a zinc finger protein fused to a dimerizing peptide preferentially bind to the substrate relative to phage displaying a zinc fusion protein fused to a nondimerizing peptide. The phage that bind to the substrate are isolated. A segment of the genome of a phage binding to the substrate is sequenced to determine the identity of a dimerizing peptide. In some such methods, the first and second binding sites are in opposing orientations in the substrate. In some methods, the phage displaying a zinc finger protein fused to ~~the~~ a dimerizing peptide bind to the substrate via display of two copies of the zinc finger protein and the dimerizing peptide, whereby the two copies of the zinc finger protein respectively bind to the first and second binding sites, and the two copies of the dimerizing peptide bind to each other. In some methods, the peptide is a random peptide. In some methods, the peptide is 30 amino acids or fewer in length.--

Please amend the paragraph beginning on line 6 of page 10 as follows:

--One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information ~~(http://www.ncbi.nlm.nih.gov/)~~. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to

calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).--

Please amend the paragraph beginning on line 8 of page 11 as follows:

--The application provides methods for selecting dimerization peptides that mediate association of linked functional proteins domains. ~~Th~~ The peptides can mediate such association by homodimerizing with each other, by heterodimerizing with the linked protein domains, or by binding to an entity, such as a DNA target site, itself bound by the linked protein domains. In particular, such peptides are useful for mediating association of complexes of multiple zinc finger proteins thereby affording greater specificity and/or affinity in binding of the zinc finger proteins to proximately spaced target segments.--

Please amend the paragraph beginning on line 18 of page 17 as follows:

-- Zinc finger proteins are often expressed with a heterologous domain as fusion proteins. Common domains for addition to the ZFP include, e.g., transcription factor domains (activators, repressors, co-activators, co-repressors), silencers, oncogenes (e.g., myc, jun, fos, myb, max, mad, rel, ets, bcl, ~~myb,~~ mos family members etc.); DNA repair enzymes and their associated factors and modifiers; DNA rearrangement enzymes and their associated factors and modifiers; chromatin associated proteins and their modifiers (e.g. kinases, acetylases and deacetylases); and DNA modifying enzymes (e.g., methyltransferases, topoisomerases, helicases, ligases, kinases, phosphatases, polymerases, endonucleases) and their associated factors and modifiers. A preferred domain for fusing with a ZFP when the ZFP is to be used for repressing expression of a target gene is a the KRAB repression domain from the human KOX-1 protein (Thiesen et al., *New Biologist* 2, 363-374 (1990); Margolin et al., *Proc. Natl. Acad. Sci. USA* 91, 4509-4513 (1994); Pengue et al., *Nucl. Acids Res.* 22:2908-2914 (1994); Witzgall et al., *Proc. Natl. Acad. Sci. USA* 91, 4514-4518 (1994). Preferred domains for achieving activation include the HSV

VP16 activation domain (see, e.g., Hagmann et al., *J. Virol.* 71, 5952-5962 (1997)) nuclear hormone receptors (see, e.g., Torchia et al., *Curr. Opin. Cell. Biol.* 10:373-383 (1998)); the p65 subunit of nuclear factor kappa B (Bitko & Barik, *J. Virol.* 72:5610-5618 (1998)and Doyle & Hunt, *Neuroreport* 8:2937-2942 (1997)); Liu et al., *Cancer Gene Ther.* 5:3-28 (1998)), or artificial chimeric functional domains such as VP64 (Seifpal et al., *EMBO J.* 11, 4961-4968 (1992)).--

Please amend the paragraph beginning on line 26 of page 22 as follows:

--ZFP polypeptides, dimerizing peptides linked to the same, and nucleic acids encoding fusion proteins of ZFPs and dimerizing peptides can be made using routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook et al., *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel et al., eds., 1994)). In addition, nucleic acids less than about 100 bases can be custom ordered from any of a variety of commercial sources, such as The Midland Certified Reagent Company (mcrc@oligos.com), The Great American Gene Company ~~(http://www.genco.com)~~, ExpressGen Inc. ~~(www.expressgen.com)~~, Operon Technologies Inc. (Alameda, CA). Similarly, peptides can be custom ordered from any of a variety of sources, such as PeptidoGenic (pkim@ccnet.com), HTI Bio-products, inc. ~~(http://www.htibio.com)~~, BMA Biomedicals Ltd (U.K.), Bio.Synthesis, Inc.--

Please amend the paragraph beginning on line 27 of page 29 as follows:

-- **Phage Display Libraries.** Phagemid vectors used in the selections were created from pZif12 (7) by restoring the reading frame between the Zif12-coding region and gene III and by introducing convenient restriction sites at the start of Zif12. Libraries containing randomized peptides were constructed by cassette mutagenesis, using NN(G/C/T) randomized condons for the initial libraries and NN(G/T) for the reoptimization libraries. The complete fusion protein used for phage display ~~(Fig. 1A)~~ contained a PelB signal sequence; a short leader peptide (NH_2-EPRAQNS in initial selections and NH_2-EP in reoptimizations); the random peptide; residues 4-60 of Zif268 (numbering as in ref. 8); a linker that includes an amber condom; and residues 23-424 of M13 gene III product. The ligated phagemid libraries were electroporated into XL-1 Blue

E. coli cells, yielding $\approx 10^8$ transformants for the initial selection libraries and $\approx 10^9$ transformants for each of the reoptimization libraries.--

Please amend the paragraph beginning on line 15 of page 31 as follows:

-- Labeled DNA probes were generated as follows. For the gel-shift studies ~~shown in Fig. 2B~~, oligos corresponding to the phage-selection target site (5'GGTTGCAG**TGGGCGCGCCCA**CAGTACTTGAACGTAACG-3' and 5'-CGTTACGTTCAAGTACTG**TGGGCGCGCCCA**CTGC-3', Zif12 sites in bold) or a single-site mutant (bold regions above replaced with the sequences 5'-**TGGGCG**TATGCT-3' and 5'AGCATA**CGCCCA**-3') were annealed and end-labeled with Klenow. A labeled restriction fragment was used for quantitative studies. The oligos 5'-GGAATTCCTGA-TCAAGATCTGGTCACGTCCATAGGCTAGGCATGTCAAGGCTGTATG-3' and 5'-GGGATCCACTCGCGAACGCGTCCTTGTAG**TGGGCGCGCCCA**CATACAGCCTTGACA T-3' (Zif12 sites in bold) were annealed, extended by mutually primed extension, and cloned into the *Eco*RI and *Bam*HI sites of pBluescript II SK(+). The probe was prepared by digesting the plasmid with *Eco*RI and *Not*I; labeling the DNA with Klenow, (α-^{32}P)dCTP, and (α-^{32}P)dGTP; and purifying the small fragment by native PAGE.--

Please amend the paragraph beginning on line 26 of page 32 as follows:

--To select dimerization motifs, we attached random peptides to a DNA-binding domain and selected those fusion proteins that could bind more stably to a symmetric DNA site ~~(Fig. 3)~~. Random 15- and 30-residue peptides were expressed at the amino terminus of the first two zinc fingers of Zif268 (8, 17) (we refer to this two-finger peptide as Zif12), and these peptide-Zif12 fusions were displayed on filamentous bacterio-phage. Phage from the 15- and 30-mer libraries, representing 10^8 different sequences from each library, were pooled, and our affinity-selection protocol was used with a target DNA duplex containing an inverted repeat of the Zif12-binding site. The original Zif12 peptide, which lacks any N-terminal extension, binds specifically, but weakly, to the "half-site" sequence TGGGCG, and Zif12 phage are not retained by the target DNA. Therefore, our protocol enriches for phage that display peptides that augment the DNA-binding activity of the zinc fingers.—

IN THE CLAIMS

Please amend claims 5 and 6 as follows.

5. (Amended) A zinc finger complex, comprising two or more fusion proteins, each fusion protein [a first fusion protein] comprising a [first] zinc finger protein and a [first] peptide linker [and a second fusion protein comprising a second zinc finger protein and a second peptide linker], wherein the [first and second] fusion proteins are [complexed] joined to each other by specific binding of the [first and second] peptide linkers, and wherein the [first and second] peptide linkers are nonnaturally occurring peptides.

6. (Amended) The zinc finger complex of claim 5, wherein the [first and second] peptide linker [linker are first and second copies of] of each fusion protein has the same [linker] sequence.

CURRENTLY PENDING CLAIMS

1-4. Withdrawn.

5. (Amended) A zinc finger complex, comprising two or more fusion proteins, each fusion protein comprising a zinc finger protein and a peptide linker, wherein the fusion proteins are joined together by specific binding of the peptide linkers, and wherein the peptide linkers are nonnaturally occurring peptides.

6. (Amended) The zinc finger complex of claim 5, wherein the peptide linker of each fusion protein is the same.

7-19. Withdrawn.

20. (New) The zinc finger complex of claim 5, wherein the zinc finger protein of each fusion protein has the same sequence.